Journal of Biomechanics 45 (2012) 1753–1758

Contents lists available at SciVerse ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Validation of vibration testing for the assessment of the mechanical properties of human lumbar motion segments

S.J.P.M. van Engelen [a,b], M.H.M. Ellenbroek [c], B.J. van Royen [a,d], A. de Boer [c], J.H. van Dieën [a,b,*]

[a] Research Institute MOVE, VU University Amsterdam, Van der Boechorststraat 9, NL-1081 BT Amsterdam, The Netherlands
[b] Faculty of Human Movement Sciences, VU University Amsterdam, The Netherlands
[c] Department of Applied Mechanics, University of Twente, Enschede, The Netherlands
[d] Department of Orthopaedic Surgery, VU University Medical Centre, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Accepted 8 May 2012

Keywords:
Biomechanics
Human lumbar spine
Experimental modal analysis
Validation

ABSTRACT

Experimental modal analysis is a non-destructive measurement technique, which applies low forces and small deformations to assess the integrity of a structure. It is therefore a promising method to study the mechanical properties of the spine in vivo. Previously, modal parameters successfully revealed artificially induced spinal injuries. The question remains however, whether experimental modal analysis can be applied successfully in human spinal segments with mechanical changes due to physiological processes. Since quasi-static mechanical testing is considered the "gold standard" for assessing intervertebral stiffness, the purpose of our study was to examine if the mechanical properties derived from vibration testing and quasi-static testing correlate.

Six cadaver human spines (L1–L5) were loaded quasi-statically in bending and torsion, while an optical system measured the angular rotations of the individual motion segments. Subsequently, the polysegmental spines were divided into L2–L3 and L4–L5 segments and a shaker was used to vibrate the upper vertebra, while its response was obtained from accelerometers in anteroposterior and mediolateral directions. From the resulting frequency response function the eigenfrequencies (ratio between stiffness and mass) and vibration modes (pattern of motion) were determined.

The vibration results showed clear eigenfrequencies for flexion–extension (mean 121.83 Hz, SD 40.05 Hz), lateroflexion (mean 132.17, SD 34.80 Hz) and axial rotation (mean 236.17 Hz, SD 81.45 Hz). Furthermore, the correlation between static and dynamic tests was significant ($r=0.73$, $p=0.01$). In conclusion, the findings from this study show that experimental modal analysis is a valid method to assess the mechanical properties of human lumbar motion segments.

1. Introduction

The spinal column is a complex mechanical structure that protects the neural structures while allowing movement in six degrees of freedom. With degeneration or injury this mechanical structure starts to fail; mechanical testing showed that the intervertebral stiffness decreases with increasing degeneration until the final phase when the stiffness of the motion segment increases again due to secondary tissue responses such as osteophyte formation (Al-Rawahi et al., 2011). Although mechanical dysfunction due to alterations in spinal segment stiffness is thought to be a major cause of low back pain (Panjabi, 1992; Van Dieën et al., 2003), currently available methods to examine local segmental stiffness such as quasi-static mechanical tests can only be performed ex-vivo. In vivo, intervertebral stiffness is derived from degeneration estimates on MRI and radiographic images, which have low correlations with macroscopic grading of degeneration and mechanical test results (Benneker et al., 2005; Quint and Wilke, 2008).

A non-destructive measurement technique that is used in engineering to examine the mechanical properties of structures and can be used to identify damage, such as cracks in aeroplane wings, is experimental modal analysis. Modal tests measure the response of a system to an applied dynamic load. After fast Fourier transformation, the resulting frequency response function (FRF) allows determination of modal parameters such as eigenfrequencies (ratio between stiffness and mass), vibration modes (pattern of motion) and damping. Since only low forces and small deformations are necessary to obtain the FRF, experimental modal analysis might also have clinical utility, for example in locating the presence and severity of structural disruption of the human skeletal system, as was shown by Cornelissen et al. (1986;

* Corresponding author at: Research Institute MOVE, VU University, Van der Boechorststraat 9, NL-1081 BT Amsterdam, The Netherlands.
Tel.: +31 20 59 88501; fax: +31 20 4448529.
E-mail addresses: j.h.van.dieen@vu.nl, j_h_van_dieen@fbw.vu.nl (J.H. van Dieën).

0021-9290
http://dx.doi.org/10.1016/j.jbiomech.2012.05.009

1987) and Christensen et al. (1986) who performed several studies on the stiffness of the human tibia and the use of modal parameters to monitor fracture healing.

Vibration testing has also been used to study structural disruption of the spine. Kasra et al. (1992) found that removal of posterior elements slightly decreased the eigenfrequency, although not significantly, and Kawchuk et al. (2008) found significant changes in the FRF data before and after successive disc transection and mounting of spinal instrumentation. Van Engelen et al. (2011) showed that the eigenfrequencies of goat spinal motion segments for flexion–extension, lateroflexion and axial rotation can be determined by modal testing, and that it is possible to relate specific structural alterations (annulus puncture) to specific changes of the modal parameters (lower eigenfrequency for axial rotation). Although these results are encouraging, the examinations were carried out in healthy spinal segments in which the integrity of the disc was artificially altered. It is unsure whether the induced changes are representative for the stiffness changes with degeneration. Moreover, "natural" intervertebral disc degeneration involves multiple changes such as endplate fractures, stiffening of the annulus fibrosis, the appearance of annular tears and loss of water content of the nucleus pulposus (Adams and Roughley, 2006). The complex variety of degenerative phenomena might adversely affect the reliability of the FRF and the theoretical relationship between stiffness and eigenfrequency. For example, with degeneration it might no longer be valid to use a linear approximation for the system, even for small deflections. Furthermore, due to changing damping characteristics, the resonance peaks in the FRF might become very small or the peaks might overlap, making it difficult to distinguish the individual modes of vibration. Also, changing damping characteristics might change the location of the peak in the FRF and thus affect the eigenfrequency. Since the results from dynamic experiments have never been correlated to the results from "gold standard" static experiments, it is not certain that experimental modal analysis is a valid and reliable method to quantify segmental stiffness of spines with mechanical changes due to physiological processes.

The purpose of this study was to establish whether a significant and positive correlation exists between static stiffness values and eigenfrequencies, which would indicate that experimental modal analysis is a valid method to obtain information about spinal segment stiffness.

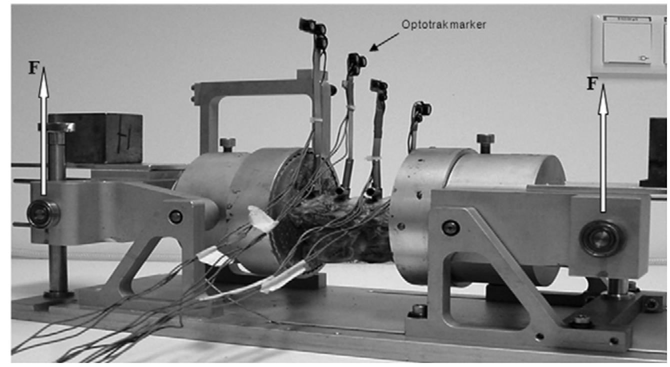

Fig. 1. Custom made device for static measurements. The arrows indicate where the force was applied by the material testing system to ensure pure bending moments. The specimen was rotated 90° to shift between flexion–extension and lateral bending. For axial rotation the left side with the cup was pulled by a small steel cable driven by the same mechanical testing system. Kinematic data was recorded with an opto-electronic system (Optotrak, Northern Digital, Ontario, Canada). Infrared LED markers were rigidly fixed to the anterior surface of the vertebral bodies.

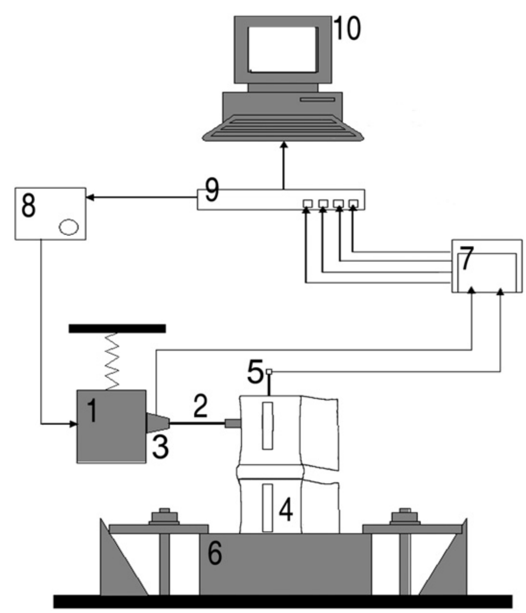

Fig. 2. Measurement set-up with shaker (1); stinger (2); force transducer (3); specimen (4); accelerometer (5); cup in which the specimen was embedded (6); conditioning amplifier (7); power amplifier (8); digital signal processing system (9) and PC (10).

2. Methods and materials

2.1. Specimens

Six human spines were harvested (5 × male and 1 × female) from cadavers with a mean age of 75 years (range 65–88 years) at the time of death. Lumbar sections from L1–L5 were examined with magnetic resonance imaging (MRI; Siemens Symphony 1.5 T: Syngo MR A30, Berlin, Germany) to exclude gross deformities and syndesmoses. Subsequently, the musculature and ligaments were carefully removed. The spines were first tested quasi-statically and thereafter with modal testing.

2.2. Quasi-static testing

Prior to mechanical testing, the upper (L1) and lower end vertebrae (L5) were embedded in cups fitting the mechanical testing machine using a low melting temperature alloy (Cerrolow-147; 48% bismuth, 25.6% lead, 12% tin, 9.6% cadmium and 4% indium). The polysegmental spines were then placed in a custom-made measuring device (Fig. 1), previously described by Busscher et al. (2010). The measuring device was driven by a hydraulic material testing system (model 8872, Instron & IST, Norwood, Canada). A compression load of 250 N was applied to the spines for 1 h. Subsequently, the compression load was removed and 10 continuous load cycles from −4 N m to +4 N m at a rate of 0.5°/s were applied in three loading directions: right and left lateral bending (LB), flexion–extension (FE) and right and left axial rotation (AR). The load and loading rate were kept relatively low to guarantee that the spines were not damaged during testing. The measuring device assured that all segments experienced equal moments; therefore, differences in deformation of neighbouring segments were determined by differences in mechanical properties only.

During mechanical testing the angular displacements were recorded with an opto-electronic system (Optotrak, Northern Digital, Ontario, Canada). Clusters of three infrared LED markers were rigidly fixed to the anterior surface of the vertebral bodies and were related to the anatomical axes of motion of the specimens. This was done by making a short recording while pointing at the motion axes with a probe that contained six markers.

2.3. Experimental modal analysis

After quasi-static testing, the polysegmental spines were dissected in the monosegmental specimens L2–L3 and L4–L5. L3 was potted as previously described, and L5 remained embedded. Two screws were inserted in the top vertebra perpendicular to the endplate for later accelerometer attachment.

A schematic drawing of the measurement set-up is presented in Fig. 2. Vibration was provided by an electromagnetic vibration exciter or shaker

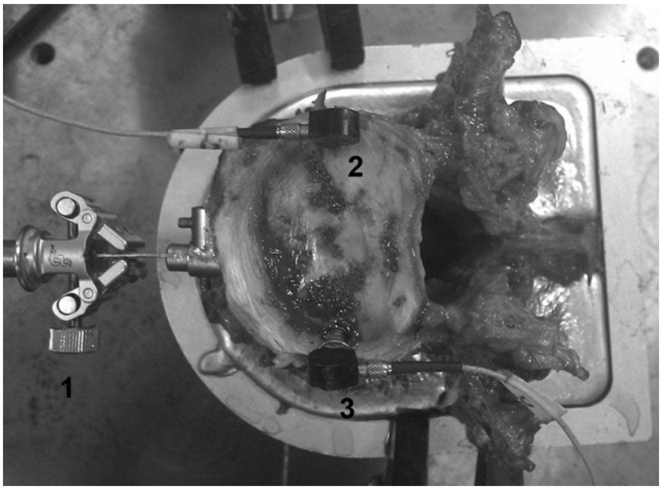

Fig. 3. Top view of the monosegmental specimen with the clamp (1) and accelerometers (2 and 3) attached to the vertebra.

(Brüel & Kjær, type 4809) suspended from elastic bands. The shaker was attached to the anterior surface of the vertebral bodies where previously the infrared LED markers were fixed. A stinger (flexible steel rod) was placed in between the clamp and the shaker to ensure that the motions of the top vertebra were unhindered by the shaker and that no shear forces and moments were applied. The applied vibration force was quantified by an impedance head (Brüel & Kjær, type 8001) placed in between the shaker and the stinger and the response was collected by uniaxial accelerometers (Brüel & Kjær, type 4393), each weighing 1 g. The excitation signal was provided by a digital signal processing system (Siglab, model 20-42) that was connected to a laptop computer. The excitation signal was current driven amplified (Brüel & Kjær, type 2706) and consisted of random frequencies between 0 and 1 kHz. The applied vibration force and the response from the accelerometers were amplified (Brüel & Kjær, type Condition Amplifier NEXUS, 4-channel) and fed back to the Siglab system. The measured data were sampled at a rate of 5120 samples/s.

The measurement protocol consisted of three parts: first the excitation was applied at the midline of the vertebra in the anteroposterior (AP) direction and also the response was measured in the AP-direction by attaching the accelerometers with bee wax to the screws in the top vertebra (Fig. 3). Second, the excitation was applied at the same location, but under an angle of 90°, thereby exciting in the mediolateral (ML) direction. The response was measured in the ML-direction by changing the direction of the accelerometers on the screws. Third, the excitation remained the same since it also excited AR, but one accelerometer was attached to the facet joint surface in the ML-direction and one accelerometer was attached to the anterior side of the vertebra in the ML-direction.

During measurements, the coherence of input and output signal (with values between 0 and 1) was available as feedback on the monitor. When the coherence was <0.8 the measurement was considered of insufficient quality and the trial was repeated. Response linearity was verified by varying the input force and noting a proportional increase in output level and by surveying the coherence.

3. Data analysis

3.1. Quasi-static tests

Kinematic data from the quasi-static tests were extracted using a computer program written in Matlab (Mathworks, Natick MA, USA). The neutral zone stiffness (k_{NZ}) was assessed conform the method of Smit et al. (2011) which makes use of a double sigmoid function that is fitted over the raw load–deflection data to filter noise and to allow for an analytical calculation of the non-linear segment compliance. The region with the highest compliance represents the neutral zone (NZ) and its boundaries were determined by assessment of the maximum and minimum of the second derivative. k_{NZ} was calculated as the slope of the NZ (Fig. 4). When the NZ could not be determined from the second derivative, the stiffness was calculated as the slope of the tangent at zero load. For this study, only the trials for which the correlation between measurement data and the fitted curve was

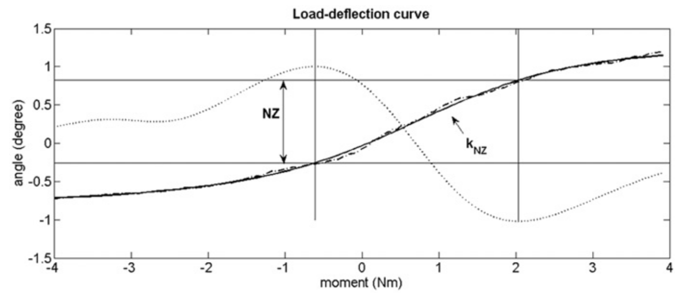

Fig. 4. Typical load–deflection curve from the static bending test. The dash-dotted line represents the actual measurement data, the solid line is the curve fit and the dotted line is the 2nd derivative of the curve fit. The neutral zone (NZ) is based on the maximum and minimum in the 2nd derivative, the neutral zone stiffness (k_{NZ}) is calculated as the slope of the NZ.

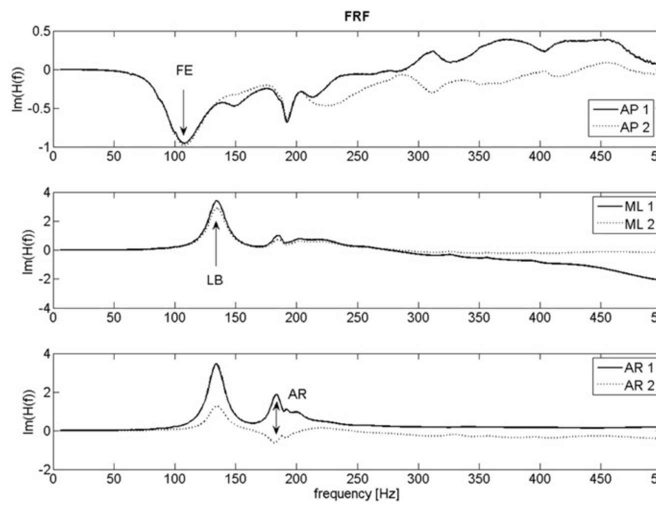

Fig. 5. Typical frequency response function (FRF) from the vibration test. The upper panel shows the frequency response from the two accelerometers in the anteroposterior direction (AP1–2), the middle panel from the accelerometers in the mediolateral direction and the bottom panel shows the results when axial rotation is excited. In this example the mode shapes for flexion–extension (FE) and lateral bending (LB) appear around 110–130 Hz, and axial rotation (AR) can be observed at 180 Hz.

≥ 0.90 were considered eligible. k_{NZ} was calculated for AR, FE and LF as the mean value over the eligible measurement cycles.

3.2. Modal tests

The FRF was extracted from Siglab using a computer program written in Matlab (Mathworks, Natick MA, USA). In connection with the 90° phase criterion, the imaginary part of the FRF displays a peak when the excitation frequency equals the eigenfrequency; therefore, the imaginary part of the FRF was plotted versus the frequency. The first peak in the FRF-plot for the ML-measurement represents the eigenfrequency for LB, and the first peak in the FRF-plot for the AP-measurement represents the eigenfrequency for FE. Anti-phase motion between two accelerometers on either side of the longitudinal axis indicates that a mode shape is a torsion mode; therefore the first bipolar peak in the AR-measurement represents the eigenfrequency for AR (Fig. 5).

The eigenfrequencies with coinciding mode shapes LB, FE and AR were also analysed in ME'scope VES 5.0 (Vibrant Technology, Scotts Valley CA, USA). With this program, a 3D structure model from a motion segment was build and animated with the frequency response data to see how the motion segment moved at a specific frequency. With the animated model the eigenfrequencies derived from the FRF-plots were confirmed.

3.3. Correlation between static test results and vibration test results

The relation between eigenfrequencies, stiffness and mass is expressed as: $2\pi f_i = \sqrt{k_i/m_i}$, where f_i is the eigenfrequency (in Hz), k_i the generalised stiffness (in N m/rad) and m_i the generalised (angular) mass (in kg m^2). The indices (i=1, 2, …, n) indicate the individual degrees of freedom of the system. Since the correlation coefficient expresses the *linear* relation between two parameters, the eigenfrequencies were multiplied by 2π and squared before calculating the correlation between eigenfrequencies and stiffness. The correlation was tested statistically by calculating the second order partial correlation coefficient with segment level (L2–L3 and L4–L5) and loading direction (LB, FE and AR) as control variables. All statistical tests were performed using SPSS 16 (SPSS INC., Chicago, IL), and significance was set at $p<0.05$.

4. Results

In all spines quasi-static mechanical testing and modal testing was successful, except in spine 3 for which the correlation between the measured data and the fitted load–deflection curve was below 0.90. From the data in Table 1, it can be seen that the eigenfrequencies for LB, FE and AR show the same pattern as k_{NZ}; when k_{NZ} was the lowest for LB and the highest for AR, also the eigenfrequencies were the lowest for LB and the highest for AR. This can be observed for all spines, except for spine 2 in which the value for LB was derived from calculating the slope of the tangent at zero load. It is also interesting to see that for the L2–L3 segments the eigenfrequencies for LB were lower than for FE (except in spine 2), but that for the L4–L5 segments the opposite can be observed. Due to missing data from spine 3 it is unclear whether this was also the case for the static tests. Of course, more spines are needed to test whether this difference between L2–L3 and L4–L5 is related to structural differences (e.g. moment of inertia) between the levels. The pattern described above is replicated by the scatter plot in Fig. 6. Also, statistical testing revealed that the correlation between the eigenfrequencies and k_{NZ} was significant (with outliers, r=0.58, p=0.04; without outliers, r=0.73, p=0.01).

Table 1
Quasi-static stiffness (k_{NZ}) and the eigenfrequencies for the spines in the three loading directions.

		k_{NZ} [Nm/°]			Eigenfrequencies [Hz]		
		LB	FE	AR	LB	FE	AR
Spine 1	**L2–L3**	0.31	0.53	2.24	104	138	240
Spine 2	**L2–L3**	2.97[a]	1.42	2.56	134	106	183
Spine 3	**L2–L3**	[b]	[b]	[b]	170	187	331
Spine 4	**L4–L5**	1.28[c]	0.57	2.07	128	110	188
Spine 5	**L4–L5**	0.74	0.22	1.32	85	66	140
Spine 6	**L4–L5**	1.67	0.73	2.61	172	124	335

LB, lateral bending; FE, flexion–extension; AR, axial rotation.

[a] k_{NZ} calculated from the slope of the tangent at zero load.
[b] Data discarded due to low correlation between measured and curve fitted data.
[c] k_{NZ} calculated from 9 instead of 10 loading cycles.

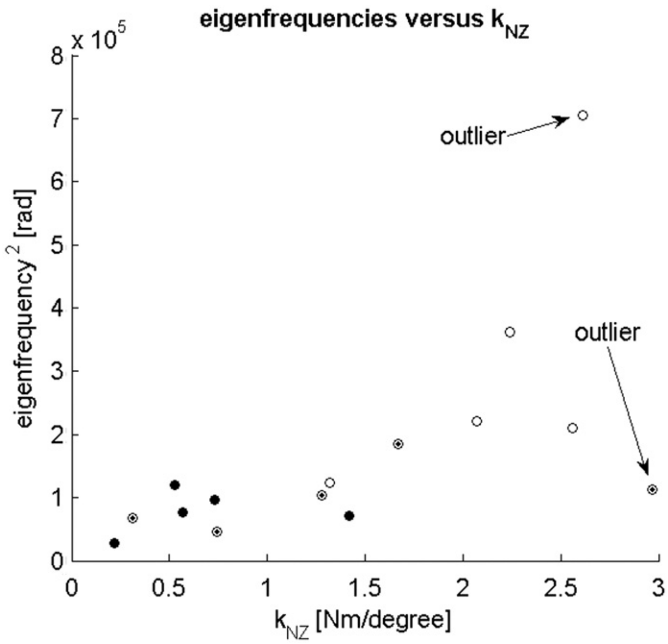

Fig. 6. Scatterplot of the eigenfrequencies versus neutral zone stiffness (k_{NZ}). The circles with a dot represent lateral bending, filled circles represent flexion–extension and open circles represent axial rotation. The correlation between the eigenfrequencies and k_{NZ} was significant (with outliers, r=0.58, p=0.04; without outliers, r=0.73, p=0.01).

5. Discussion

The present study was designed to examine if experimental modal analysis can be used to assess the mechanical properties of human lumbar spines. Previously, modal parameters succesfully revealed artificially induced structural alterations in human, goat and pig spinal segments (Kasra et al., 1992; Kawchuk et al., 2008, 2009; Van Engelen et al., 2011). However, the question remained whether modal parameters can also be obtained succesfully in human spines with mechanical changes due to physiological processes. Since degeneration might affect the theoretical relationship between stiffness and eigenfrequency, experimental modal analysis might not be a valid method to quantify spinal segment stiffness. To our knowledge, this was not yet established empirically. This study was the first to show that quasi-static mechanical test results which are considered the "gold standard" for assessing intervertebral stiffness, are correlated to the mechanical properties as derived from vibration testing.

Already in 1972, Kazarian addressed several factors that might influence the validity of dynamic test results. First, the testing machine together with the specimen forms a very complex dynamical system and it is uncertain to what extent this influences the motion response of the motion segment. Second, the assumption of constant characteristics (stiffness, damping, and geometry) might not be met in dynamic testing. Third, the system might show (partial) nonlinear behaviour. In short, modal analysis relies on quite a range of assumptions which, if incorrect, may invalidate the parameters that are calculated from the measurement outcomes, while not all assumptions can be checked beforehand. Therefore, each "new" measurement method has to be validated against a gold standard. In this study, improper assumptions might have influenced the eigenfrequencies that were obtained from peak detection in the FRF (e.g. homogeneous mass distribution, linearity). However, also k_{NZ} is calculated from measurement outcome and is subject to assumptions (e.g. fixed loading rate). It is unlikely that a perfect correlation can be found between two inherently different methods. Still, the correlation coefficient that was found in this study between quasi-static testing and dynamic testing was 0.73, which indicates that the model assumptions were not violated and that experimental modal analysis is a valid way of obtaining information on intervertebral stiffness. Moreover, the good agreement between static and dynamic test results indicates that eigenfrequencies, like static stiffness values, can be used to rank severity of mechanical changes. It was already shown that modal testing is capable of discriminating between different artificial structural alterations; the results from the present study showed that modal testing is also able to discriminate between physiological changes in spinal mechanics.

When examining the agreement between methods it is important to realise that differences in measurement set-up will generate different results. This became clear from previous studies that used numerous set-ups to test the kinematics of the lumbar spine. First, testing at higher loading rates will generate higher stiffness values due to the viscoelastic properties of spinal motion segments (Race et al., 2000). Although the loading rate was different between static and dynamic testing, this difference will only have created an offset and will not have influenced the correlation between quasi-static and modal test results. Second, it is important that the segments can move unhindered by the testing apparatus, since coupling between motion directions is the normal kinematic behaviour (Panjabi et al., 1994). To assure that the motion segments could move unhindered during both testing conditions, polysegmental spinal sections were used for quasi-static testing and during the dynamic tests constrictions were prevented by using a stinger to connect the shaker to the top vertebra. A third methodological difference between static and dynamic testing is the influence of mass. In the dynamic tests, the mass and the distribution of mass of the vibrating part of the motion segment affect the outcomes. An increase in mass at constant stiffness lowers the eigenfrequency, since the eigenfrequency is the ratio between stiffness and mass. In static testing however, mass does not play a role. In the present study, the mass of the vibrating segment could not be measured because the segments had to remain intact for further experiments. Also in patients, obtaining segmental mass (and inertial properties) is difficult, although it might be approximated by assessment of density calibrated quantitative computed tomography images (Van Kuijk et al., 1990a,b). Finally, the limitation of always having to perform quasi-static testing prior to modal testing might potentially have caused an order effect. Although the spines were sprayed with a 0.9% saline solution during preparation and testing to keep the segments moist, an offset between the static and dynamic results might have resulted from dehydration. Additionally, although the loads were kept low, it is possible that spinal tissues sustained some damage during quasi-static testing.

Clearly, none of the techniques presented here can at present be performed in the living human. We have only just begun to tackle the issues that are involved in making this method applicable for per-operative use. Progress is made in small steps; in our previous study, we assessed the sensitivity and reliability of experimental modal analysis with the simplest scenario: healthy, in vitro, isolated monosegmental specimens with no complicating contributions of the surrounding tissue (Van Engelen et al., 2011). In this study isolated, aged human motion segments were tested in a controlled in-vitro condition, to examine the validity of modal analysis of the human spine. Although the grade of degeneration was not investigated in this study, it is likely that the segments that were used would have had a range of degenerative alterations. It would have been of interest to obtain a wider range of mechanical properties by testing younger specimens as well, but availability is limited. Furthermore, future studies are needed that test the spine in situ to investigate the influence of the upper and lower body on the modal parameters and a methology needs to be developed that is able to assess the location and severity of degenerated segments in otherwise healthy spines.

In conclusion, this study found a significant and positive correlation between static stiffness values and eigenfrequencies and therefore we conclude that experimental modal analysis is a reliable and valid method to quantify intervertebral stiffness.

Conflict of interest statement

There are no financial and personal relationships with other people or organisations that inappropriately biased this work and no funding was received.

Acknowledgements

The authors would like to thank A.G.M. Wolbert, Dr. A.J. Van Der Veen, Dr. I. Kingma and A. Bisschop for their efforts during collection and analysis of the data.

References

Adams, M.A., Roughley, P.J., 2006. What is intervertebral disc degeneration, and what causes it? Spine 31 (18), 2151–2161.

Al-Rawahi, M., Luo, J., Pollintine, P., Dolan, P., Adams, M.A., 2011. Mechanical function of vertebral body osteophytes, as revealed by experiments on cadaveric spines. Spine 36 (10), 770–777.

Benneker, L.M., Heini, P.F., Anderson, S.E., Alini, M., Ito, K., 2005. Correlation of radiographic and MRI parameters to morphological and biochemical assessment of intervertebral disc degeneration. European Spine Journal 14 (1), 27–35.

Busscher, I., Van der Veen, A.J., Van Dieën, J.H., Kingma, I., Verkerke, G.J., Veldhuizen, A.G., 2010. In vitro biomechanical characteristics of the spine: a comparison between human and porcine spinal segments. Spine 35 (2), E35–E42.

Christensen, A.B., Ammitzbøll, F., Dyrbye, C., Cornelissen, M., Cornelissen, P., Van der Perre, G., 1986. Assessment of tibial stiffness by vibration testing in situ—I. Identification of mode shapes in different supporting conditions. Journal of Biomechanics 19 (1), 53–60.

Cornelissen, M., Cornelissen, P., Van der Perre, G., Christensen, A.B., Ammitzboll, F., Dyrbye, C., 1987. Assessment of tibial stiffness by vibration testing in situ—III. Sensitivity of different modes and interpretation of vibration measurements. Journal of Biomechanics 20 (4), 333–342.

Cornelissen, P., Cornelissen, M., Van der Perre, G., Christensen, A.B., Ammitzbøll, F., Dyrbye, C., 1986. Assessment of tibial stiffness by vibration testing in situ—II. Influence of soft tissues, joints and fibula. Journal of Biomechanics 19 (7), 551–561.

Kasra, M., Shirazi-Adl, A., Drouin, G., 1992. Dynamics of human lumbar intervertebral joints. Experimental and finite-element investigations. Spine 17 (1), 93–102.

Kawchuk, G.N., Decker, C., Dolan, R., Carey, J., 2009. Structural health monitoring to detect the presence, location and magnitude of structural damage in cadaveric porcine spines. Journal of Biomechanics 42 (2), 109–115.

Kawchuk, G.N., Decker, C., Dolan, R., Fernando, N., Carey, J., 2008. The feasibility of vibration as a tool to assess spinal integrity. Journal of Biomechanics 41 (10), 2319–2323.

Kazarian, L., 1972. Dynamic response characteristics of the human vertebral column. An experimental study on human autopsy specimens. Acta Orthopeadica Scandinavia, 1–186.

Panjabi, M.M., 1992. The stabilizing system of the spine. Part II. Neutral zone and instability hypothesis. Journal of Spinal Disorders 5 (4), 390–396. discussion 397.

Panjabi, M.M., Oxland, T.R., Yamamoto, I., Crisco, J.J., 1994. Mechanical behavior of the human lumbar and lumbosacral spine as shown by three-dimensional load–displacement curves. Journal of Bone Joint Surgery America 76 (3), 413–424.

Quint, U., Wilke, H.J., 2008. Grading of degenerative disk disease and functional impairment: imaging versus patho-anatomical findings. European Spine Journal 17 (12), 1705–1713.

Race, A., Broom, N.D., Robertson, P., 2000. Effect of loading rate and hydration on the mechanical properties of the disc. Spine 25 (6), 662–669.

Smit, T.H., Van Tunen, M.S., Van der Veen, A.J., Kingma, I., Van Dieën, J.H., 2011. Quantifying intervertebral disc mechanics: a new definition of the neutral zone. BMC Musculoskeletal Disorders 12, 38.

Van Dieën, J.H., Cholewicki, J., Radebold, A., 2003. Trunk muscle recruitment patterns in patients with low back pain enhance the stability of the lumbar spine. Spine 28 (8), 834–841.

Van Engelen, S.J.P.M., Van der Veen, A.J., De Boer, A., Ellenbroek, M.H., Smit, T.H., Van Royen, B.J., Van Dieën, J.H., 2011. The feasibility of modal testing for measurement of the dynamic characteristics of goat vertebral motion segments. Journal of Biomechanics 44 (8), 1478–1483.

Van Kuijk, C., Grashuis, J.L., Steenbeek, J.C., Schütte, H.E., Trouerbach, W.T., 1990a. Evaluation of postprocessing dual-energy methods in quantitative computed tomography. Part 1. Theoretical considerations. Investigative Radiology 25 (8), 876–881.

Van Kuijk, C., Grashuis, J.L., Steenbeek, J.C., Schütte, H.E., Trouerbach, W.T., 1990b. Evaluation of postprocessing dual-energy methods in quantitative computed tomography. Part 2. Practical aspects. Investigative Radiology 25 (8), 882–889.